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Confirmation of *Melastoma dendrisetosum* (Melastomataceae) as a distinct species based on morphological and micromorphological data

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Abstract: *Melastoma dendrisetosum* C. Chen (Melastomataceae) was first reported in 1983. Chen & Renner (2007) merged it within *M. sanguineum*. In order to clarify the difference between the two and the taxonomic status of *M. dendrisetosum*, a field investigation, herbarium survey, and an examination of morphological and micromorphological characteristics was performed. *M. dendrisetosum* was confirmed as a distinct species, obviously different from *M. sanguineum*. This species is narrowly endemic and extremely endangered. The authors provide the latest morphological description of *M. dendrisetosum*, aiming to lay a foundation for the precise identification and future conservation of this species.

Keywords: *Melastoma dendrisetosum*, distinct species, evidence, latest morphological description, Hainan

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基于形态与微形态分析确认枝毛野牡丹（野牡丹科）系一独立的种

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摘要: 枝毛野牡丹 (*Melastoma dendrisetosum*) 属于野牡丹科野牡丹属, 首次发表于 1983 年, 而 *Flora of China* (2007) 则将其归入了毛菍 (*M. sanguineum*)。为弄清二者的区别及枝毛野牡丹的分类地位, 本文作者采用野生地实地考察、各大标本馆标本比较鉴定、引种地植株形态测定以及微形态扫描电镜观察, 现已证实: 枝毛野牡丹是明显区别于毛菍的一个独立的植物种, 该种自然分布区极其狭窄, 正处于极度濒危的生存状态。作者根据自己多年的科研积累对枝毛野牡丹进行了最新的形态描述, 旨在为正确识别、积极保护该物种奠定基础。

关键词: 枝毛野牡丹, 独立物种, 证据, 最新形态描述, 海南

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Introduction

The genus *Melastoma* Linn. (Linnaeus, 1753) (Melastomataceae) comprises approximately 100 species distributed from Southern Asia to the Northern part of Oceania and the Pacific Islands (Chen, 1983, 1984; Meyer, 2001), but only 22 species have been recognized in the *Flora of China* (Chen & Renner, 2007), and 31 new species in Borneo were published by Wong (Wong, 2016); he believed that there are 80–90 species in *Melastoma*. In fact, understanding of this genus is not detailed enough to ensure the exact number of species in it (Huang et al., 2018), and there may thus be other new species yet to be discovered.

Nine species and one variety in China were reported in the revision of the Chinese *Melastoma* in 1983 (Chen, 1983), wherein the new species *M. dendrisetosum* was only reported to be distributed in Hainan and was similar to *M. penicillatum*. This treatment was followed by the same author in *Flora Reipublicae Popularis Sinicae* (Chen, 1984). However, in *Flora of China* (Chen & Renner, 2007) Chen and Renner followed Meyer's (Meyer, 2001) revision and recognized only five Chinese taxa and incorporated *M. dendrisetosum* and *M. penicillatum* into *M. sanguineum*. However, subsequent studies indicated that *M. penicillatum* was a distinct species from both morphological characteristics and molecular results (Chao et al., 2014); Huang et al.'s research indicated that *M. dendrisetosum* was an independent species mainly based on the difference in the indumentum of the hypanthium and the molecular results (2018); however, there are other morphological differences between the two, and the taxonomic status of *M. dendrisetosum* also needs to be further assessed from multiple perspectives.

Leaves are one of the important vegetative organs of plants, and are also one of the important bases for plant species identification (Zeng et al., 2017). In plant taxonomic studies, leaf characteristics are next only to the those of flowers and fruits (Wang et al., 2012; Li et al., 2010; Yang et al., 2016). The leaf epidermis of plants has certain genetic stability, and thus, the micromorphological characteristics of leaf epidermis are of important research value for interspecies or intergeneric classification (Zeng et al., 2017) and the microscopic morphological characteristics of leaves have been increasingly used in the study of modern plant taxonomy and in the classification and identification of difficult groups (Peng et al., 2011; Xu et al., 2013; Yang et al., 2016). Epidermal hair is the most common appendage on plant leaves, and its distribution characteristics and morphological characteristics are important means of plant identification and taxonomic study (Baran & Zdemir, 2009; Wang et al., 2015). Epidermal hair is confirmed as a classification feature with applied value for interspecies classification (Yang et al., 2016). Stomata are the subsidiary structure of leaf epidermis and are evolved from epidermal cells during long-term development. The density of stomatal organs is also different among species, and can be used as the basis of species classification (Zeng et al., 2017). The results of previous studies have proved that sporopollen is an important basis for plant classification (Shao & Fan, 2003). The morphological characteristics of flower pollens are controlled genetically and have strong stability and genetic conservatism, which are not easily affected by environmental factors. Pollen grain size, shape, and outer wall ornamentation can be used to study plant classification (Erdtman, 1978; Xu, 2007). When the classical morphological method proves difficult to solve the problem of classification, study of pollen morphology and surface ornamentation often plays a very valuable role (Zhang & Zhou, 1998; Zhang et al., 2001; Zhang, 2004; Yan & Li, 2003a, b; Zhang et al., 2014; Hong et al., 2015; Li et al., 2017).

Thus, in order to clarify the difference between *M. dendrisetosum* and *M. sanguineum* and the taxonomic and survival status of *M. dendrisetosum*, we made several botanical trips to Hainan. Along with morphological observation and comparative identification, micromorphological scanning electron microscopy was performed to evaluate the species status of *M. dendrisetosum* and to lay the foundation for its further protection and utilization.

1 Materials and methods

1.1 Materials

The morphological data of the species described here are based on living plants growing in the Diaoluo Mountain Nature Reserve and introduced from there to grow in Fujian Agriculture and Forestry University (FAFU), and specimens collected in Diaoluo Mountain Nature Reserve, Hainan (Table 1) were deposited at major herbariums such as HITBC, IBK, IBSC, and KUN.

Table 1 Specimens, locality, and vouchers

Species	Locality	Voucher
<i>Melastoma dendrisetosum</i>	Diaoluo Mountain Nature Reserve, Hainan	Peng 201206
<i>M. sanguineum</i>	Diaoluo Mountain Nature Reserve, Hainan	Peng 201204

1.2 Methods

1.2.1 Morphological observation and conservation status investigation

Morphological observation The morphological traits of *M. dendrisetosum* and *M. sanguineum* are based on living plants, growing in the wild or in the campus of FAFU. We selected 30 individuals of *M. dendrisetosum* and *M. sanguineum* each that had grown in the wild, and 10 from the campus of FAFU and observed them with naked eyes; the whole plants or some organs were photographed with a digital camera (Canon H G 10) and the specimens introduced in 1.1 were observed and compared carefully.

Conservation status investigation Several scientific trips were made to the wild native places recorded in previous literature such as Luohui (currently Qionghai), Baoting, Wanning, and Lingshui to investigate the distribution of *M. dendrisetosum* in the wild and its survival status.

1.2.2 Micro-morphology observations by scanning electron microscope (SEM)

Leaf stomata micro-morphology observation by SEM According to Guo's method (Guo, 2006), fresh mature leaves of *M. dendrisetosum* and *M. sanguineum* were cut separately into 5 mm × 5 mm square blocks near the midrib; the blocks were quickly dipped in formal-aceto-alcohol (FAA) fixative solution for 2 h at room temperature, then stepwise dehydrated twice with 30%, 50%, 70%, 85%, 95%, and 100% ethanol, each stage was dehydrated for 15 min. The blocks were dried using the critical point drying method and were fixed on copper tables, coated with the JFC-1200 ion sputter coater, and observed and photographed under the JSM-5310LV SEM.

Feature description terminologies on stoma in this article are used with reference to Zhang & Zhuang (2004), Zhang et al. (2013) and Zeng et al. (2017).

Pollen micro-morphology observation by SEM The anthers of long stamens of *M. dendrisetosum* and *M. sanguineum* were collected, stored in a dust-free place, and naturally dried for 48–72 h to collect the pollens. The pollens were bonded on copper tables using double-sided

tape, coated with the JFC-1200 ion sputter coater, and observed and photographed under the JSM-5310LV SEM. Ten pollen grains of each of the two species were randomly selected to measure the length of the polar axis p and the equatorial axis e , respectively.

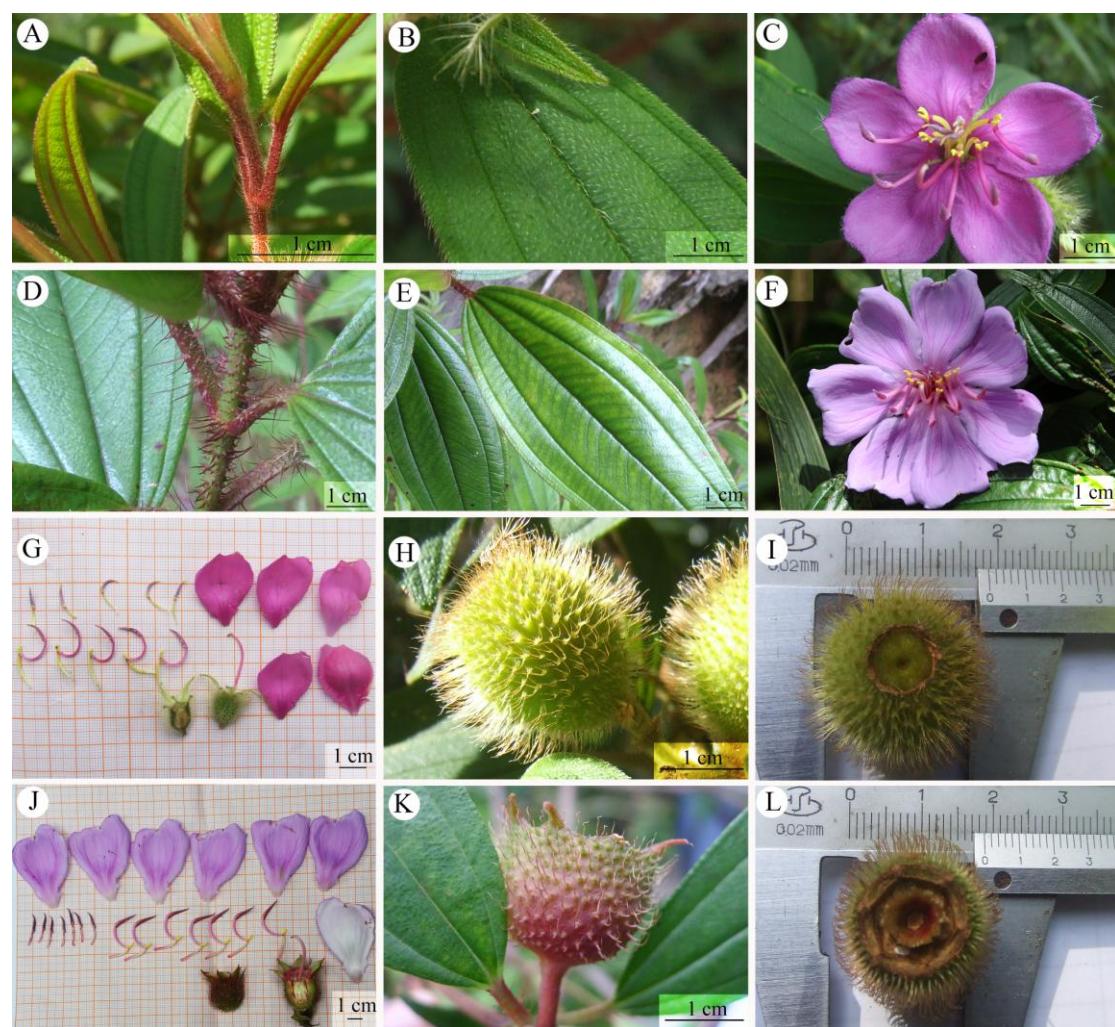
Feature descriptions on pollen shape, size, and exine ornamentation in this article are in reference to Li et al. (2017).

2 Results

2.1 Obvious different morphological traits between *M. dendrisetosum* and *M. sanguineum*

The obvious different morphological traits between *M. dendrisetosum* and *M. sanguineum* are shown in Fig. 1 and Table 2.

According to our investigation, a few traits such as plant height, oblique branchlets, and seed color are slightly similar between *M. dendrisetosum* and *M. sanguineum*, but rather obvious differences are present in many traits including the flower, fruit, stem, and leaf characteristics, especially the flower and fruit traits (Fig. 1, Table 2). Moreover, the flowering time of the two species is also different, *M. dendrisetosum* blooms from March to April whereas *M. sanguineum* blooms throughout the year.



Note: A-C, G-I. *M. dendrisetosum*; D-F, J-L. *M. sanguineum*; A, D. Stem and petiole; B, E. Leaf; C, F, G, J. Opening flowers; H, K. Flower anatomy; I, L. Fruit.

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Fig. 1 Photographs indicating the obvious difference in some main morphological traits between *M. dendrisetosum* and *M. sanguineum*

Table 2 Comparison of the main morphological traits of *M. dendrisetosum* and *M. sanguineum*

sanguineum		
Items	<i>M. dendrisetosum</i>	<i>M. sanguineum</i>
Stem	Color	Red purple
	Branch diameter	0.1-0.2 cm
	*Epidermal hair	White puberulous
Leaf	Blade size	9-10 × 4-5 cm
	*Midvein	Sunken and with white coarse bristles
	*Blade epidermal hair	Rough and hairy
	*Blade margin	With bristly ciliate
	*Petiole	0.4-0.7 cm length, with white sericeous puberulous
Flower	Flower diameter	4-6 cm
	*Petal Number	5
	*Petal color	Both adaxially and abaxially rose-red
	*Petal apex	Sharp-pointed and with a bunch of prickles
	*Petal margin	With shortly ciliate
Fruit	*Stamen Number	10 including 5 long stamens and 5 short stamens
	*Filament color	Pale yellow
	*Hypanthium	Epidermal with densely white basal-branching bristles
	*Shape	Urceolate and truncated
	Size	Diameter 1.5-1.9 cm
Fruit	*Epidermis	Green epidermal with densely white basal-branching bristles
	*Calyx tube mark on the top of fruit	Narrow and Circular
	*The top of the fruit	Near flat, with slightly sunken style mark in the center
		Rounded protruded end of ovary, with obvious sunken near hexagonal style mark in the center

Note: *indicates the obvious different traits. The same below.

2.2 Obviously different micro-morphological traits of stomata and pollen between *M. dendrisetosum* and *M. sanguineum*

The obvious different micro-morphological traits of stomata and pollen between *M. dendrisetosum* and *M. sanguineum* are shown in Fig. 2 and the micro-morphological comparison of their stoma and pollen are shown in Table 3.

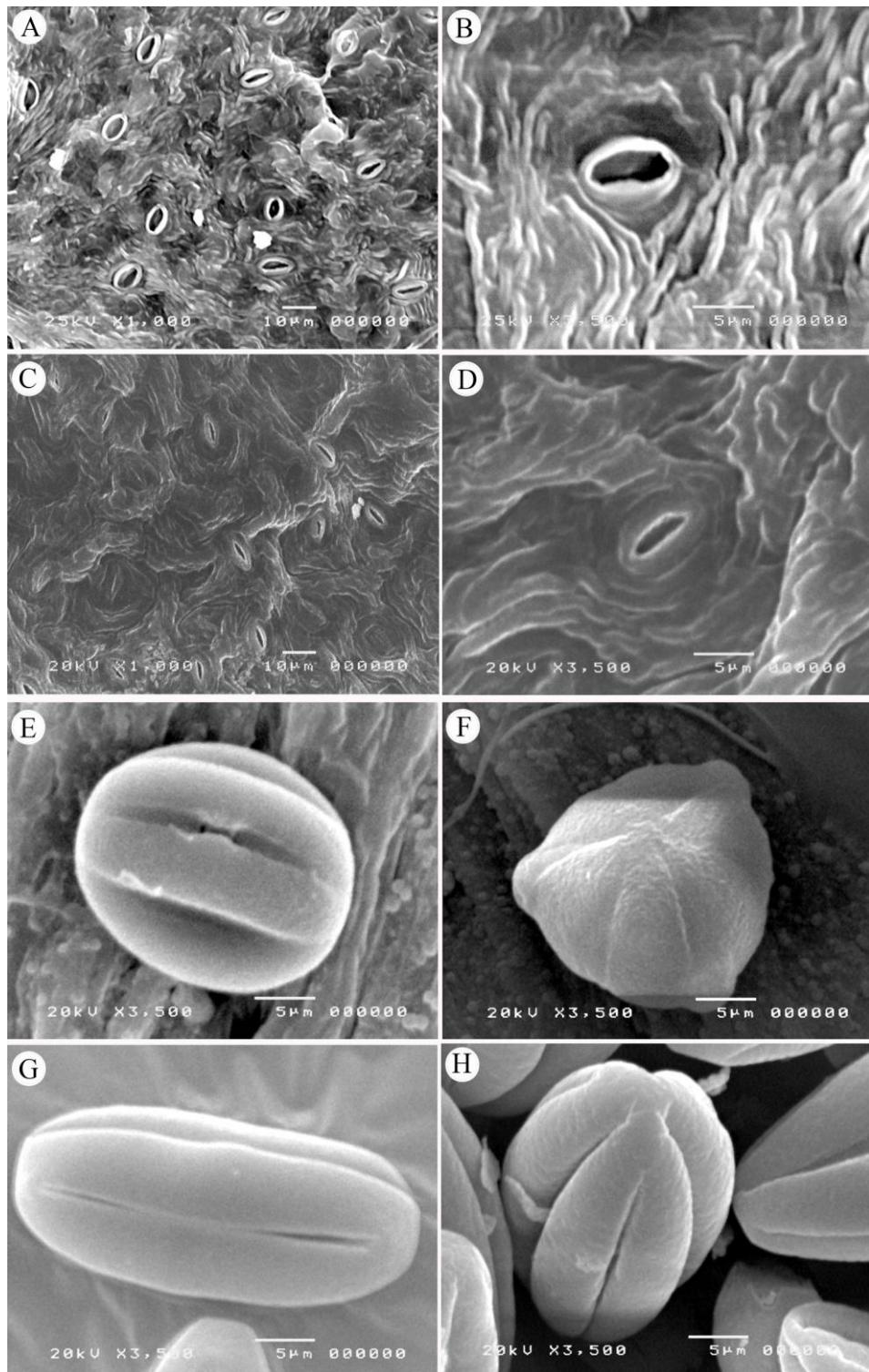
Table 3 Comparison of stomata and pollen micro-morphology between *M. dendrisetosum* and *M. sanguineum*

<i>M. sanguineum</i>		
Items	<i>M. dendrisetosum</i>	<i>M. sanguineum</i>
Stoma	Shape of stomatal complex	L/W=1.52, elliptic
	*Opening degree	Larger
	Sunken degree of stomatal complex relative to epidermal cells	Sunken but not obvious
	*Stomatal outer arch cover	Narrow, obvious uplift
	Ornamentation of stomatal outer arch cover	Nearly smooth
	*Inner margin of stomatal outer arch cover	Obvious thickening and sinuate
	*Peripheral ornamentation	More slender and uplift wave stripes
	*Length of long axis	22.60 μ m
	*Type	Middle pollen
	*Polar axis length (P) \times	22.60 \times 21.23 μ m
Pollen	Equatorial axis length (E)	
	*Equator view	Long sphere with furrows
	*Polar view	Six-lobed circular with 6 slight furrows
	*Exine ornamentation	Densely covered with wormlike projections
		Densely and evenly covered with shallow holes

Note: According the ratio of long/wide, elliptic. $1.35 < L/W < 1.55$; Oblong elliptic. $L/W > 1.55$; Widely elliptic. $L/W < 1.35$ (Zeng et al., 2017).

Although the micro-morphology of the stomata in *M. dendrisetosum* and *M. sanguineum* shows similar shape of the stomatal complex and ornamentation of the stomatal outer arch cover, obvious differences are observed in the opening degree, the sunken degree of stomatal complex relative to epidermal cells, stomatal outer arch cover and its inner margin, and the peripheral ornamentation of the stomatal complex (Fig. 2: A-D, Table 3).

Although the micro-morphology of pollen of *M. dendrisetosum* and *M. sanguineum* is similar in the 6 germinating furrows, obvious difference are observed in some aspects such as pollen size (including length of the long axis and $P \times E$), equator and polar view, and exine ornamentation (Fig. 2: E-H, Table 3).



Note: **A, B.** The stoma of *M. dendrisetosum*; **C, D.** The stoma of *M. sanguineum*; **E, F.** Pollen equatorial and polar view of *M. dendrisetosum*; **G, H.** Pollen equatorial and polar view of *M. sanguineum*.

Fig. 2 Photographs demonstrating the micro-morphology of stomata and pollen from *M. dendrisetosum* and *M. sanguineum*

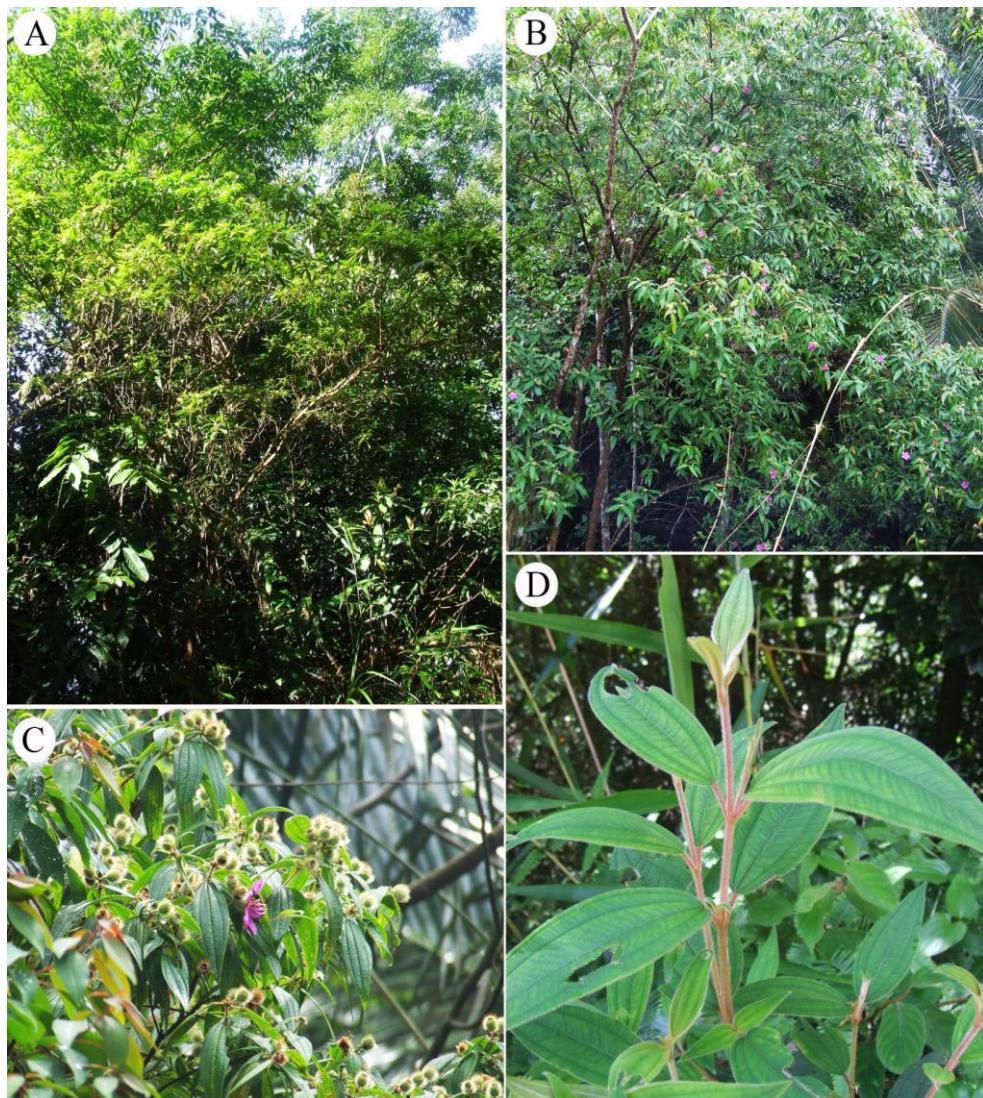
2.3 Conservation status of *M. dendrisetosum*

M. dendrisetosum is only found in a valley of the Diaoluo Mountain National Nature Reserve in April 2012, distributed at an altitude of 350-400 meters, and is scattered with a total of less than 300 individuals at nearly 100 kilometers from its type locality Luohui (currently Qionghai), Hainan, China (Fig. 3). This entity is a shrub of 2 to 3 meters tall, and grows in slightly shaded places on forest margins, roadsides, or stream sides. The photographs of its habitat and branches are shown in Fig. 4. Other species found growing nearby this species include *Sapium discolor*, *Mallotus japonicas*, *Passiflora foetida*, and so on. In addition, although *M. dendrisetosum* and *M. sanguineum* were found to grow together in Diaoluo Mountain National Nature Reserve, the authors have not found any intermediate between them in the wild.



Note: Qionghai (previously known as Luohui) is the type locality of *M. dendrisetosum*.

Fig. 3 Location of Diaoluo Mountain National Nature Reserve



Note: **A, B.** Wild habitat; **C.** Branches with many fruits and flowers; **D.** Branch only with leaves.

Fig. 4 Photographs indicating the habitat or branches of *M. dendrisetosum*

3 Discussion and Conclusion

3.1 Discussion

3.1.1 *M. dendrisetosum* is a distinct species

Melastoma dendrisetosum was recognized as a distinct species when it was first published by Chen (Chen, 1983), but was incorporated into *M. sanguineum* by Meyer (Meyer, 2001) and the *Flora of China* (Chen & Renner, 2007), rather than being treated as an independent species. It is well known that the characteristics of flowers and fruits are important for plant classification. In this study, the authors carefully compared the main differences in some characteristics of the flower and fruit between *M. dendrisetosum* and *M. sanguineum*, and found some morphological traits unaffected by environmental change, such as the petal tip and the accessory hair on it, the number of petals and stamens, fruit type and color, the mark of calyx tube, and so on, which are obviously different. These differences in flower and fruit characteristics indicate that *M.*

dendrisetosum is a species distinct from *M. sanguineum*.

In this study, the authors found that *M. dendrisetosum* and *M. sanguineum* are obviously different in some leaf traits as the midvein, blade epidermal hair, blade margin, and petiole and some stoma traits such as stoma opening degree, stomatal outer arch cover, inner margin of stomatal outer arch cover, and stoma peripheral ornamentation.

The present study indicated that *M. dendrisetosum* and *M. sanguineum* are obviously different in some pollen traits such as length of long axis, type, polar and equatorial axis length, and the equatorial and polar view.

These observations strongly confirmed that *M. dendrisetosum* is a species significantly different from *M. sanguineum* in the epidermal hairs of leaves, stomata, and pollen micromorphology. In addition, a recent molecular level perspective also indicated that *M. dendrisetosum* should be considered a distinct species (Huang et al., 2018).

3.1.2 *M. dendrisetosum* is an endangered species

In April 2012, the authors investigated the living condition of *M. dendrisetosum* and found that it is only sporadically distributed in a valley of the Diaoluo Mountain National Nature Reserve, at an altitude of 350-400 m, with a total of less than 300 individuals at nearly 100 kilometers from its type locality, Luohui (currently Qionghai), Hainan, China. According to the IUCN Red Categories and Criteria (IUCN, 2001), *M. dendrisetosum* satisfies the criteria B1 (i.e., extent of occurrence, 100 km²) and B2 (i.e., present only in one location and declining quality of habitat). Moreover, the author's previous research (Peng et al., 2014) found that there is no self-compatible self-pollination and agamospermy in this species, and that the breeding system of *M. dendrisetosum* is a typical facultative inbred type with a relative reproductive success (RRS) of 0.035, which is perhaps one of the main reasons for its endangered status.

Moreover, in 2016, Huang et al. (2018) found that the habitat of *M. dendrisetosum* in the area was degraded further and that the number of mature individuals was declining, and they strongly believe that *M. dendrisetosum* would be categorized as 'Critically Endangered'. Therefore, the habitat of the species should first be fully protected, and any destruction to it must be strictly prohibited; secondly, measures of habitat protection combined with introduction of propagation protection should be taken to expand the population.

3.1.3 New morphological description of *M. dendrisetosum* should be prepared

So far, the morphological description on *M. dendrisetosum* that can be found is still only from Chen (Chen, 1984). In the past three decades, the morphological and micro-morphological characteristics of this species have been revealed in a stepwise manner, with continuous investigation and studies. Thus, it is necessary to supplement the original description and prepare a new description in order to facilitate the correct understanding and identification this species.

3.2 Conclusion

3.2.1 *M. dendrisetosum* is a distinct species in an endangered living status

Morphological trait observation results showed that *M. dendrisetosum* is obviously different from *M. sanguineum* in some traits of the flower, fruit, branch, and leaf as well as in the flowering date. Micro-morphological trait observation by SEM indicated some obvious differences between

M. dendrisetosum and *M. sanguineum* in the traits of stoma as well as pollen. Thus, *M. dendrisetosum* is a distinct species. Based on the resource investigations of the genus *Melastoma*, only one clump of *M. dendrisetosum* was found in Diaoluo National Nature Reserve, with less than 300 individuals left, indicating that this species is in a critically endangered living status and should be protected actively and carefully. Botanical trips to Hainan and our observations of this species at that location will facilitate a better understanding of this species, and help us to determine why it is restricted to such a small area.

3.2.2 Redescription of *M. dendrisetosum*

Melastoma dendrisetosum C. Chen in Journ. South China, Agri. Coll. 4 (1): 35, fig.10-12, 1983; C. Chen in Fl. Reip. Popul. Sin. 53(1) 168, 1984.

Type: China, Hainan, Lohwei (Luohui), orient. Hainan Exp. 89 (ANT). Specimens cited: Hainan, Wenning, I Chung 4016 (IBSC); Hainan, Lingshui, C. Wang 36782 (IBSC); Hainan, Wannin, Z. X. Li 628676 (IBSC); Hainan, Wannin, F. W. Xin 634549 (IBSC); Hainan, Wannin, F. W. Xin 634103 (IBSC); Hainan, Diaoluo Z. X. Li 522642 (IBSC); Hainan, Wannin, F. W. Xin 634549 (IBSC); Hainan, Wannin, Y. Zhong 316567 (IBSC); Hainan, Xinlong, 317381 (IBSC); Hainan, Jiaziling, 299256 (IBSC); Hainan, Baoting, S. Q. Chen 199964 (IBSC); Hainan, Linshui, Z. Huang 70921 (IBSC); Hainan, Diaoluo National Nature Reserve, D. H. Peng et al. 201203 (FAFU); Hainan, Diaoluo National Nature Reserve, D. H. Peng et al. 201204 (FAFU); Hainan, Diaoluo National Nature Reserve, D. H. Peng et al. 201206 (FAFU).

Shrub, 2-3 m. Stems terete, densely long sericeous and puberulous with branches; leaf blade stiffly papery, lanceolate to oblong-lanceolate, apex acuminate, cuneate or obtuse at base, 4.5-8.5 × 1.7-3 cm, secondary veins 2 on each side of midvein, margin entire or densely shallow-denticulate, bristly ciliate, adaxially strigose, abaxially densely villous and puberulent. Inflorescence terminal, umbellate, very short, subcapitiate, 3-4 flowered, base with 2 foliaceous bracts, smaller than leaves; peduncle densely long sericeous and puberulous, ca. 0.2 cm. Hypanthium densely white branching setae, ca. 1 cm. Calyx lobes linear-triangular, ca. 0.6 cm long, covered with branching setae, with a small lobe, ca. 0.2 cm long between them. Petals rose red, rhombic-obovate, oblique above middle, ca. 2.5 × 2 cm, with a bundle of bristles at apex, with shortly ciliate. Stamens heteromorphic, longer stamens ca. 3.6 cm, anthers ca. 2.4 cm, purplish red, connectives extended at base, curved, bases with 2 tubercles, filaments slightly longer than extended connection; shorter stamens ca. 1.7 cm, anthers ca. 0.8 cm, with connection not extended, bases with 2 tubercles. Ovary half-inferior, densely setose. Fruit urceolate when young, truncate, apex covered with bristles. Persistent hypanthium covered with branching bristles. Capsule diameter ca. 1.5-1.9 cm. Flowering in March – April, and **fruiting in May – August**.

Distribution and Habitat: *M. dendrisetosum* is only found in Diaoluo Mountain National Nature Reserve, Hainan, China. It grows in slightly shaded places on forest margins, roadsides, or stream sides between 350-400 m, and tends to grow in areas that receive 5 to 6 hours of sunlight a day. Other species found growing nearby this species include *Sapium discolor*, *Mallotus japonicas*, *Passiflora foetida*, etc.

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